

strain, wherein at least one of the F1 progeny that carry the dominant allele also carry at least one random mutation;

backcrossing gametes from male F1 progeny to at least one female of the index inbred strain, with or without the index allele, to obtain N2 backcross progeny, wherein at least one of the N2 backcross progeny that carry the dominant allele also exhibit the outlying phenotype; and

verifying that the outlying phenotype is caused by a segregating single point mutation.

2. A method as claimed in Claim 1 wherein any of the crosses employ preserved gametes.

3. A method as claimed in Claim 1 wherein the F1 progeny and some of the N2 progeny exhibit an extreme outlying phenotype.

4. A method as claimed in Claim 3 wherein the segregating mutation is a heterozygous modifier of the index phenotype selected from a group consisting of an enhancing modifier and a suppressing modifier.

5. A method as claimed in Claim 1 wherein the dominant allele is a *Min* allele at an *Apc* locus in a mouse.

6. A method as claimed in Claim 1 wherein the index inbred strain and the founder inbred strain share an isogenic genetic background.

7. A method as claimed in Claim 6 further comprising the step of mapping the segregating mutation using a mapping partner strain produced by the steps of:

treating an animal of an index strain with a mutagenic agent to induce point mutations in the treated animal;

crossing the treated animal to an animal of the index strain to produce F1 progeny; and

sib-mating F1 and subsequent generation progeny until detrimental and lethal mutations are eliminated.

8. A method as claimed in Claim 1 wherein the founder inbred mouse strain is produced by a method comprising the step of treating a wild-type inbred mouse with a mutagenic agent to induce point mutations.

9. A method as claimed in Claim 8 wherein the mutagenic agent is ethylnitrosourea.

26. A method as claimed in Claim 6 wherein the method identifies a segregating mutation at a genetic locus that modifies tumor multiplicity in a C57BL/6 mouse congenic for the *Min* allele at the *Apc* locus, the method comprising the steps of:

outcrossing at least one male C57BL/6 mouse carrying random point mutations to a female C57BL/6 mouse congenic for the *Min* allele at the *Apc* locus to obtain F1 progeny, wherein at least one of the F1 progeny carries both the *Min* allele and a random point mutation; and

backcrossing gametes from male F1 progeny to at least one female C57BL/6 mouse congenic for the *Min* allele at the *Apc* locus to obtain N2 backcross progeny, wherein at least one of the N2 backcross progeny carries the *Min* allele and has a tumor multiplicity that is modified relative to tumor multiplicity in a C57BL/6 mouse congenic for the *Min* allele at the *Apc* locus, the modified tumor multiplicity being characteristic of the segregating mutation.

Sub e4 27. (Amended) A method as claimed in Claim 26 wherein the modified tumor multiplicity is evaluated according to a method comprising the steps of:

repeatedly applying for random permutations of mice among N2 backcross subkindreds a likelihood ratio test of the null hypothesis that no multiplicity modifier is segregating to obtain a p-value, wherein a p-value of less than 0.05 indicates a potential carrier of the segregating mutation;

when the p-value is less than 0.05, calculating, for each potential carrier that has offspring with information about tumor multiplicity, a LOD score for presence of the segregating mutation, wherein the LOD score is \log_{10} of a ratio of the probability of offspring phenotype data if the potential carrier mouse carries a multiplicity modifier to the probability of offspring phenotype data if the potential carrier mouse does not carry a multiplicity

modifier, and wherein the denominator probabilities are calculated from an estimated background distribution and the numerator probabilities are calculated from a mixture of the estimated background distribution and an estimated modified distribution, where the estimated distributions are obtained by the method of maximum likelihood; and ranking the LOD scores of the potential carriers, whereby animals having the highest LOD scores are likely carriers of the segregating mutation.

28. (Amended) A method as claimed in claim 26, further comprising the step of mapping the segregating mutation in the N2 backcross progeny using a mapping partner strain having the genetic background of the index inbred strain.

29. A method as claimed in Claim 28 wherein the mapping partner strain is produced by the steps of:

treating a C57BL/6 mouse with a mutagen to introduce random point mutations;
crossing the treated mouse to a C57BL/6 mouse to produce F1 progeny; and
sib-mating F1 and subsequent generation progeny until detrimental and lethal mutations are eliminated.

30. (New) A method for identifying a segregating single point mutation at a genetic locus that modifies an index phenotype in a mouse index inbred strain, the segregating mutation causing an outlying phenotype relative to the index phenotype, the method comprising the steps of:

outcrossing at least one male animal of a mouse founder inbred strain to at least one female animal of a mouse index inbred strain to obtain F1 progeny, the founder inbred strain carrying random point mutations relative to a wild-type animal of the founder inbred strain, the index inbred strain carrying a congenic dominant allele at a locus known to confer the index phenotype and being genetically distinguishable from the founder inbred strain, wherein at least one of the F1 progeny that carry the dominant allele also carry at least one random mutation;

backcrossing gametes from male F1 progeny to at least one female of the index inbred strain, with or without the index allele, to obtain N2 backcross progeny, wherein at least one of the N2 backcross progeny that carry the dominant allele also exhibit the outlying

phenotype; and

verifying that the outlying phenotype is caused by a segregating single point mutation.

31. (New) A method as claimed in Claim 30 wherein any of the crosses employ preserved gametes.

32. (New) A method as claimed in Claim 30 wherein the F₁ progeny and some of the N2 progeny exhibit an extreme outlying phenotype.

33. (New) A method as claimed in Claim 32 wherein the segregating mutation is a heterozygous modifier of the index phenotype selected from a group consisting of an enhancing modifier and a suppressing modifier.

34. (New) A method as claimed in Claim 30 wherein the dominant allele is a *Min* allele at an *Apc* locus in a mouse.

35. (New) A method as claimed in Claim 30 wherein the index inbred strain and the founder inbred strain share an isogenic genetic background.

36. (New) A method as claimed in Claim 35 further comprising the step of mapping the segregating mutation using a mapping partner strain produced by the steps of:
treating an animal of an index strain with a mutagenic agent to induce point mutations in the treated animal;
crossing the treated animal to an animal of the index strain to produce F1 progeny;
and
sib-mating F1 and subsequent generation progeny until detrimental and lethal mutations are eliminated.

37. (New) A method as claimed in Claim 30 wherein the founder inbred mouse strain is produced by a method comprising the step of treating a wild-type inbred mouse with a mutagenic agent to induce point mutations.

38. (New) A method as claimed in Claim 37 wherein the mutagenic agent is ethylnitrosourea.

39. (New) A method as claimed in Claim 35 wherein the method identifies a segregating mutation at a genetic locus that modifies tumor multiplicity in a C57BL/6 mouse congenic for the *Min* allele at the *Apc* locus, the method comprising the steps of:

outcrossing at least one male C57BL/6 mouse carrying random point mutations to a female C57BL/6 mouse congenic for the *Min* allele at the *Apc* locus to obtain F1 progeny, wherein at least one of the F1 progeny carries both the *Min* allele and a random point mutation; and

backcrossing gametes from male F1 progeny to at least one female C57BL/6 mouse congenic for the *Min* allele at the *Apc* locus to obtain N2 backcross progeny, wherein at least one of the N2 backcross progeny carries the *Min* allele and has a tumor multiplicity that is modified relative to tumor multiplicity in a C57BL/6 mouse congenic for the *Min* allele at the *Apc* locus, the modified tumor multiplicity being characteristic of the segregating mutation.

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July 16 > 40. (New) A method as claimed in Claim 39 wherein the modified tumor multiplicity is evaluated according to a method comprising the steps of:

repeatedly applying for random permutations of mice among N2 backcross subkindreds a likelihood ratio test of the null hypothesis that no multiplicity modifier is segregating to obtain a p-value, wherein a p-value of less than 0.05 indicates a potential carrier of the segregating mutation;

when the p-value is less than 0.05, calculating, for each potential carrier that has offspring with information about tumor multiplicity, a LOD score for presence of the segregating mutation, wherein the LOD score is \log_{10} of a ratio of the probability of offspring phenotype data if the potential carrier mouse carries a multiplicity modifier to the probability of offspring phenotype data if the potential carrier mouse does not carry a multiplicity modifier, and wherein the denominator probabilities are calculated from an estimated background distribution and the numerator probabilities are calculated from a mixture of the estimated background distribution and an estimated modified distribution, where the estimated distributions are obtained by the method of maximum likelihood; and

ranking the LOD scores of the potential carriers, whereby animals having the highest

LOD scores are likely carriers of the segregating mutation.

41. (New) A method as claimed in claim 39, further comprising the step of mapping the segregating mutation in the N2 backcross progeny using a mapping partner strain.

42. (New) A method as claimed in Claim 41 wherein the mapping partner strain is produced by the steps of:

treating a C57BL/6 mouse with a mutagen to introduce random point mutations;
crossing the treated mouse to a C57BL/6 mouse to produce F1 progeny; and
sib-mating F1 and subsequent generation progeny until detrimental and lethal mutations are eliminated.
